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(FILE 'HOME' ENTERED AT 09:10:29 ON 20 JUN 2003)

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     FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, CABA, PASCAL, AGRICOLA,
     LIFESCI, EMBASE, MEDLINE, BIOTECHNO' ENTERED AT 09:11:54 ON 20 JUN 2003
L2            89 S L1 AND MARITIMA
L3            60 S L2 AND (PURIF? OR ISOLAT? OR CLON?)
L4            29 DUP REM L3 (31 DUPLICATES REMOVED)
L5             1 S L4 AND (VARIANT OR MUTANT)

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=> d l4 ibib ab 1-29

L4 ANSWER 1 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:964542 CAPLUS
DOCUMENT NUMBER: 138:35298
TITLE: **Cloning**, sequence and mutagenesis of Thermotoga **maritima** carboxymethyl **cellulase** gene, bioinformatics applications and use of the recombinant enzyme in food supplement or detergent, or for modulator screening
INVENTOR(S): Short, Jay M.; Mathur, Eric J.; Lam, David E.
PATENT ASSIGNEE(S): Diversa Corporation, USA
SOURCE: PCT Int. Appl., 168 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002101078	A2	20021219	WO 2002-US18782	20020612
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003044956	A1	20030306	US 2001-880729	20010612

PRIORITY APPLN. INFO.:
US 2001-880729 A2 20010612
US 1995-518615 A3 19950823
US 1997-951889 A3 19971016
US 1999-472857 A2 19991227

AB This invention provides hydrolases, e.g., **cellulase** enzymes, polynucleotides encoding these enzymes, the use of such polynucleotides and polypeptides and methods of making and using them. In one aspect, the invention provides enzymes having carboxymethyl **cellulase** activity. **Cloning** of carboxymethyl **cellulase** gene from Thermotoga **maritima** is described, and the genomic and encoded amino acid sequences of the enzyme are disclosed. Mutagenesis of the T. **maritima** carboxymethyl **cellulase** gene and construction of transgenic non-human animals, plants and seeds is described. Bioinformatics methods and construction of nucleic acid probes and primers related to the T. **maritima** carboxymethyl **cellulase** gene are disclosed. The recombinant thermotolerant carboxymethyl **cellulase** from T. **maritima** can be used for screening of **cellulase** modulators, treating cellulose-contg. fabric, pulp or paper products, in detergents, in food supplements or for waste treatment.

L4 ANSWER 2 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:210645 BIOSIS
DOCUMENT NUMBER: PREV200200210645
TITLE: Cellulolytic enzymes from Thermotoga species.
AUTHOR(S): Liebl, Wolfgang (1)
CORPORATE SOURCE: (1) Institut fuer Mikrobiologie und Genetik, Georg-August-Universitaet, D-37077, Goettingen Germany
SOURCE: Adams, Michael W. W. [Editor]; Kelly, Robert M. [Editor]. Methods in Enzymology, (2001) Vol. 330, pp. 290-300.

Methods in Enzymology. Hyperthermophilic enzymes: Part A. print.
Publisher: Academic Press Inc. 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA.
ISSN: 0076-6879. ISBN: 0-12-182231-1 (cloth).
DOCUMENT TYPE: Book
LANGUAGE: English

L4 ANSWER 3 OF 29 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 2001379272 EMBASE
TITLE: Galactomannanases Man2 and Man5 from Thermotoga species: Growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes.
AUTHOR: Parker K.N.; Chhabra S.R.; Lam D.; Callen W.; Duffaud G.D.; Snead M.A.; Short J.M.; Mathur E.J.; Kelly R.M.
CORPORATE SOURCE: R.M. Kelly, Department of Chemical Engineering, North Carolina State University, Box 7905, Raleigh, NC 27695-7905, United States. rmkelly@eos.ncsu.edu
SOURCE: Biotechnology and Bioengineering, (5 Nov 2001) 75/3 (322-333).
Refs: 73
ISSN: 0006-3592 CODEN: BIBIAU
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The enzymatic hydrolysis of mannan-based hemicelluloses is technologically important for applications ranging from pulp and paper processing to food processing to gas and oil well stimulation. In many cases, thermostability and activity at elevated temperatures can be advantageous. To this end, the genes encoding .beta.-mannosidase (man2) and .beta.-mannanase (man5) from the hyperthermophilic bacteria Thermotoga neapolitana 5068 and Thermotoga **maritima** were **isolated**, **cloned**, and expressed in Escherichia coli. The amino acid sequences for the mannosidases from these organisms were 77% identical and corresponded to proteins with an M(r) of approximately 92 kDa. The translated nucleotide sequences for the .beta.-mannanase genes (man5) encoded polypeptides with an M(r) of 76 kDa that exhibited 84% amino acid sequence identity. The recombinant versions of Man2 and Man5 had similar respective biochemical and biophysical properties, which were also comparable to those determined for the native versions of these enzymes in T. neapolitana. The optimal temperature and pH for the recombinant Man2 and Man5 from both organisms were approximately 90.degree:C and 7.0, respectively. The presence of Man2 and Man5 in these two Thermotoga species indicates that galactomannan is a potential growth substrate. This was supported by the fact that .beta.-mannanase and .beta.-mannosidase activities were significantly stimulated when T. neapolitana was grown on guar or carob galactomannan. Maximum cell densities increased by at least tenfold when either guar or carob galactomannan was added to the growth medium. For T. neapolitana grown on guar at 83.degree.C, Man5 was secreted into the culture media, whereas Man2 was intracellular. These localizations were consistent with the presence and lack of signal peptides for Man5 and Man2, respectively. The identification of the galactomannan degrading enzymes in these Thermotoga species adds to the list of biotechnologically important hemicellulases produced by members of this hyperthermophilic genera. .COPYRGT. 2001 John Wiley & Sons, Inc.

L4 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 2001:125828 CAPLUS
DOCUMENT NUMBER: 135:57649
TITLE: Cellulolytic enzymes from Thermotoga species

AUTHOR(S): Liebl, Wolfgang
CORPORATE SOURCE: USA
SOURCE: Methods in Enzymology (2001), 330(Hyperthermophilic Enzymes, Part A), 290-300
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB .beta.-Glucans are important natural polymers consisting of .beta.-glycosidically linked glucose residues. These polysaccharides mostly fulfill structural roles. The .beta.-1,4-glucan cellulose and mixed-linkage .beta.-1,4/1,3-glucans, such as barley .beta.-glucan and lichenan, occur as cell wall components of higher plants and lichens, and .beta.-1,3-glucans are found in the cell walls of yeast and filamentous fungi and as structural and storage polysaccharide of the marine macroalga Laminaria saccharina (laminarin). Thermostable enzymes for the degrdn. of these types of .beta.-glucans and/or their corresponding genes have been **isolated** from different strains of the heterotrophic strictly anaerobic bacterial genus Thermotoga. The following is an overview of .beta.-glucan-cleaving enzymes found in Thermotoga species and describes the prepn. and characteristics of an endoglucanase and a .beta.-glucosidase of Thermotoga **maritima**. (c) 2001 Academic Press.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:514612 SCISEARCH
THE GENUINE ARTICLE: 444GE
TITLE: **Isolation** and cell wall regeneration of protoplasts from Posidonia oceanica and Cymodocea nodosa
AUTHOR: Balestri E (Reprint); Cinelli F
CORPORATE SOURCE: Dipartimento Sci Uomo & Ambiente, Via Volta 6, I-56100 Pisa, Italy (Reprint); Dipartimento Sci Uomo & Ambiente, I-56100 Pisa, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: AQUATIC BOTANY, (JUL 2001) Vol. 70, No. 3, pp. 237-242. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0304-3770.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A method for the **isolation** of protoplasts from the seagrasses Posidonia oceanica and Cymodocea nodosa is described. **Isolation** of protoplasts was achieved using a combination of **cellulase** Onozuka R-10, hemicellulase and pectinase. **Purification** was carried out by Ficoll gradient centrifugation. A yield of 1.87 (+/- 0.16 SD) x 10(6) protoplasts per gram of fresh tissue was obtained from mesophyll cells of P. oceanica. The viability of **isolated** protoplasts was 82.5% (+/- 10.6) as confirmed by fluorescein diacetate staining. A high percentage of protoplasts of Fl oceanica (61.5 +/- 14.8%) regenerated the cell wall within 7 days as confirmed by staining with calcofluor white, but only a few protoplasts were able to divide. Callus-like structures were noticed after 20-30 days in culture. A lower yield of protoplasts, 6.9 (+/- 3.8) x 10(5) protoplasts per gram of fresh tissue, was obtained from mesophyll of C. nodosa. Viability of these protoplasts was 67.9% (+/- 12.6) after **isolation**. Some possible applications of the method are discussed. (C) 2001 Elsevier Science B.V. Ah rights reserved.

L4 ANSWER 6 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 2001:689911 CAPLUS

DOCUMENT NUMBER: 136:335862
TITLE: Identification and molecular characterization of an endoglucanase gene, celS, from the extremely thermophilic archaeon Sulfolobus solfataricus
AUTHOR(S): Limauro, Danila; Cannio, Raffaele; Fiorentino, Gabriella; Rossi, Mose; Bartolucci, Simonetta
CORPORATE SOURCE: Dipartimento di Chimica Organica e Biologica, Universita degli Studi di Napoli "Federico II", Naples, 80134, Italy
SOURCE: Extremophiles (2001), 5(4), 213-219
CODEN: EXTRFI; ISSN: 1431-0651
PUBLISHER: Springer-Verlag Tokyo
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A genomic region upstream of the alc. dehydrogenase (Ssadh) gene was **cloned** and sequenced from a library of Sulfolobus solfataricus MT4 strain. The **isolated** 4,040-bp DNA fragment revealed an open reading frame (celS), lying in the opposite direction to Ssadh, which showed significant similarity to endo-.beta.-1,4-glucanases from Pyrococcus furiosus, Thermotoga **maritima**, and T. neapolitana celS was shown to be a functional gene in vivo: a specific celS mRNA was detected by primer extension anal. showing a unique initiation transcription site coinciding with the ATG translation initiation codon. The specific gene product was detected as an extracellular **cellulase** after enzyme staining by CM-cellulose (CMC) SDS-PAGE, showing a mol. wt. in agreement with that deduced from the open reading frame. Depending on growth conditions, different levels of **cellulase** activity and specific celS transcript were detected, revealing an inductive effect of CMC and suggesting a repressive role of glucose.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
ACCESSION NUMBER: 2001:199142 BIOSIS
DOCUMENT NUMBER: PREV200100199142
TITLE: **Isolation** of leaf protoplasts from Pancratium maritimum L. and two other dune plants: Possible applications.
AUTHOR(S): Balestri, Elena (1); Luccarini, Gualtiero; Cinelli, Francesco
CORPORATE SOURCE: (1) Centro Interuniversitario di Biologia Marina ed Ecologia Applicata, P.le Mascagni 1, 57126, Livorno Italy
SOURCE: Journal of Coastal Research, (Winter, 2001) Vol. 17, No. 1, pp. 188-194. print.
ISSN: 0749-0208.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Protoplast techniques can be applied for studying physiological and biochemical processes in plant cells. In order to utilize these techniques, a method for **isolation** of viable protoplasts from dune plants was established. Using a combination of **cellulase** R-10 Onozuka, hemicellulase and pectinase protoplasts were **isolated** from younger leaves of Pancratium maritimum. Field-grown plants harvested in autumn or winter yielded approximately six times as many protoplasts per g of fresh tissue as did plants collected in spring (3.6-5.3 X 106 versus 9 X 105 protoplasts per g of fresh tissue). No protoplasts were released from plants harvested in summer. The production of protoplasts from cultivated plants reached high yields of protoplasts (3.6 X 106) independent to the season. The viability of these protoplasts was 89.2% (+-1.1). Within 2-3 days of liquid culture 88.6% (+-3.6) of the protoplasts were able to regenerate cell-walls. First divisions were

detected after 5-7 days of culture. By using the same procedure, yields of 2.31 X 106 and 1.28 X 104 protoplasts were obtained from field-collected leaves of Cakile **maritima** and Ammophila arenaria, respectively. Viability of protoplasts were 87.5% (+-4.3) and 75.1% (+-2.4), respectively. The potential applications of this method are discussed.

L4 ANSWER 8 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2000:527006 SCISEARCH
THE GENUINE ARTICLE: 331RY
TITLE: Mechanism of substrate hydrolysis by a thermophilic endoglucanase from Thermotoga **maritima**
AUTHOR: Evans B R (Reprint); Gilman A K; Cordray K; Woodward J
CORPORATE SOURCE: OAK RIDGE NATL LAB, DIV CHEM TECHNOL, OAK RIDGE, TN 37831 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOTECHNOLOGY LETTERS, (MAY 2000) Vol. 22, No. 9, pp. 735-740.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0141-5492.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 23

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A **cellulase** from the thermophile, Thermotoga **maritima** , hydrolyzed oligosaccharide substrates by an exoglucanase mode of action but acted as an endoglucanase to rapidly reduce the Viscosity of the soluble polysaccharides carboxymethylcellulose and barley beta-glucan. The V-max for hydrolysis of the substrate, p-nitrophenyl beta-D-cellobioside, was 42 mu mol min(-1) (mg protein)(-1), while that for barley beta-glucan was 637. The enzyme had little activity on crystalline cellulose.

L4 ANSWER 9 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:184666 BIOSIS
DOCUMENT NUMBER: PREV200000184666
TITLE: The mechanism of **cellulase** action on cotton fibers: Evidence from atomic force microscopy.
AUTHOR(S): Lee, Ida (1); Evans, Barbara R.; Woodward, Jonathan
CORPORATE SOURCE: (1) Electrical Engineering Department, University of Tennessee, Knoxville, TN, 37996-2100 USA
SOURCE: Ultramicroscopy, (Feb., 2000) Vol. 82, No. 1-4, pp. 213-221.
ISSN: 0304-3991.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Two **cellulases** from Trichoderma reesei - an exoglucanase, CBH I, and an endoglucanase, EG II - alone and in combination were incubated with cotton fibers. The effects of the **cellulases** on the surfaces of the cotton fibers were examined by atomic force microscopy. At high magnification, the physical effects on the fibers caused by the two types of enzymes were considerably different. Treatment with CBH I resulted in the appearance of distinct pathways or tracks along the length of the macrofibril. Treatment with EG II appeared to cause peeling and smoothing of the fiber surface. In combination, their effect was observed to be greatest when both enzymes were present simultaneously. When fibers smoothed by treatment with EG II were treated subsequently with CBH I, further evidence of path way formation caused by the action of CBH I along the fibers was observed. Incubation with a **cellulase** from Thermotoga **maritima** that lacks a cellulose binding domain had no effect on the surface of cotton fibers. These images provide the first physical evidence of differences in the effect of **cellulase** components action on the surface of cotton fibers and provide evidence for

the movement or tracking of CBH I along the fibers. The first AFM image of CBH I molecules are presented.

L4 ANSWER 10 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
ACCESSION NUMBER: 2000:295957 BIOSIS
DOCUMENT NUMBER: PREV200000295957
TITLE: Carboxymethyl **cellulase** from Thermotoga **maritima**.
AUTHOR(S): Mathur, Eric J.; Lam, David E.
ASSIGNEE: Diversa Corporation
PATENT INFORMATION: US 6008032 December 28, 1999
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 28, 1999) Vol. 1229, No. 4, pp. No pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB A **purified** thermostable enzyme is derived from the eubacterium T. **maritima**. The enzyme has a molecular weight as determined by gel electrophoresis of about 35 kilodaltons and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired.

L4 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:635426 CAPLUS
DOCUMENT NUMBER: 131:271022
TITLE: Carboxymethylcellulase from Thermotoga **maritima**
INVENTOR(S): Mathur, Eric J.; Lam, David E.
PATENT ASSIGNEE(S): Diversa Corporation, USA
SOURCE: U.S., 14 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5962258	A	19991005	US 1995-518615	19950823
US 6008032	A	19991228	US 1997-951889	19971016
US 5925749	A	19990720	US 1998-66075	19980424
US 6245547	B1	20010612	US 1999-472857	19991227
US 2003044956	A1	20030306	US 2001-880729	20010612

PRIORITY APPLN. INFO.: US 1995-518615 A3 19950823
US 1997-951889 A3 19971016
US 1999-472857 A2 19991227
AB A **purified** thermostable enzyme is derived from the eubacterium T. **maritima**. The enzyme has a mol. wt. as detd. by gel electrophoresis of .apprx.35 kilodaltons and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired.
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:450916 CAPLUS
DOCUMENT NUMBER: 131:84840
TITLE: Carboxymethyl-**cellulase** and its gene from Thermotoga **maritima**
INVENTOR(S): Mathur, Eric J.; Lam, David E.
PATENT ASSIGNEE(S): Diversa Corporation, USA
SOURCE: U.S., 13 pp.

CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5925749	A	19990720	US 1998-66075	19980424
US 5962258	A	19991005	US 1995-518615	19950823

PRIORITY APPLN. INFO.: US 1995-518615 A1 19950823

AB A **purified** thermostable enzyme is derived from the eubacterium T. **maritima**. The enzyme has a mol. wt. as detd. by gel electrophoresis of .apprx.35 kDa and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired. The genomic gene sequence of the **cellulase** allows design of oligonucleotide probes.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:201560 SCISEARCH
THE GENUINE ARTICLE: 173FW
TITLE: Comparing the thermodynamic stabilities of a related thermophilic and mesophilic enzyme
AUTHOR: Beadle B M; Baase W A; Wilson D B; Gilkes N R; Shoichet B K (Reprint)
CORPORATE SOURCE: NORTHWESTERN UNIV, DEPT MOL PHARMACOL & BIOL CHEM, 303 E CHICAGO AVE, CHICAGO, IL 60611 (Reprint); NORTHWESTERN UNIV, DEPT MOL PHARMACOL & BIOL CHEM, CHICAGO, IL 60611; UNIV OREGON, INST MOL BIOL, EUGENE, OR 97403; CORNELL UNIV, BIOCHEM MOL & CELL BIOL SECT, ITHACA, NY 14853; UNIV BRITISH COLUMBIA, DEPT MICROBIOL, VANCOUVER, BC, CANADA
COUNTRY OF AUTHOR: USA; CANADA
SOURCE: BIOCHEMISTRY, (23 FEB 1999) Vol. 38, No. 8, pp. 2570-2576. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036. ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Several models have been proposed to explain the high temperatures required to denature enzymes from thermophilic organisms; some involve greater maximum thermodynamic stability for the thermophile, and others do not. To test these models, we reversibly melted two analogous protein domains in a two-state manner. E2(cd) is the **isolated** catalytic domain of **cellulase** E2 from the thermophile Thermomonospora fusca. CenA(P30) is the analogous domain of the **cellulase** CenA from the mesophile Cellulomonas fimi. When reversibly denatured in a common buffer, the thermophilic enzyme E2(cd) had a temperature of melting (T-m) of 72.2 degrees C, a van't Hoff enthalpy of unfolding (Delta H-VH) Of 190 kcal/mol, and an entropy of unfolding (Delta S-u) of 0.55 kcaV(mol.K); the mesophilic enzyme CenA(P30) had a T-m of 56.3 degrees C, a Delta H-VH of 107 kcal/mol, and a Delta S-u of 0.32 kcal/(mol.K). The higher Delta H-VH and Delta S-u values for E2(cd) suggest that its free energy of unfolding (Delta G(u)) has a steeper dependence on temperature at the T-m than CenA(P30). This result supports models that predict a greater maximum thermodynamic stability for thermophilic enzymes than for their mesophilic counterparts. This was further explored by urea denaturation, Under reducing conditions at 30 degrees C, E2(cd) had a concentration of melting (C-m) of 5.2 M and a Delta G(u) of 11.2 kcal/mol;

CenA(P30) had a C-m of 2.6 M and a Delta G(u) of 4.3 kcal/mol, Under nonreducing conditions, the C-m and he, of CenA(P30) were increased to 4.5 M and 10.8 kcal/mol at 30 degrees C; the C-m for E2(cd) was increased to at least 7.4 M at 32 degrees C. We were unable to determine a Delta G(u) value for E2(cd) under nonreducing conditions due to problems with reversibility. These data suggest that E2(cd) attains its greater thermal stability (Delta T-m = 15.8 degrees C) through a greater thermodynamic stability (Delta Delta G(u) = 6.9 kcal/mol) compared to its mesophilic analogue CenA(P30).

L4 ANSWER 14 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:531603 SCISEARCH
THE GENUINE ARTICLE: 212FU
TITLE: Extremophiles as a source of novel enzymes for industrial application
AUTHOR: Niehaus F; Bertoldo C; Kahler M; Antranikian G (Reprint)
CORPORATE SOURCE: TECH UNIV HAMBURG, INST TECH MICROBIOL, DENICKESTR 15, D-21071 HAMBURG, GERMANY (Reprint); TECH UNIV HAMBURG, INST TECH MICROBIOL, D-21071 HAMBURG, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (JUN 1999) Vol. 51, No. 6, pp. 711-729.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0175-7598.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 200

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Extremophilic microorganisms are adapted to survive in ecological niches such as at high temperatures, extremes of pH, high salt concentrations and high pressure. These microorganisms produce unique biocatalysts that function under extreme conditions comparable to those prevailing in various industrial processes. Some of the enzymes from extremophiles have already been **purified** and their genes successfully **cloned** in mesophilic hosts. In this review we will briefly discuss the biotechnological significance of extreme thermophilic (optimal growth 70-80 degrees C) and hyperthermophilic (optimal growth 85-100 degrees C) archaea and bacteria. In particular, we will focus on selected extracellular-polymer-degrading enzymes, such as amylases, pullulanases, cyclodextrin glycosyltransferases, **cellulases**, xylanases, chitinases, proteinases and other enzymes such as esterases, glucose isomerases, alcohol dehydrogenases and DNA-modifying enzymes with potential use in food, chemical and pharmaceutical industries and in environmental biotechnology.

L4 ANSWER 15 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:147095 SCISEARCH
THE GENUINE ARTICLE: 166HH
TITLE: Molecular diversity of thermophilic cellulolytic and hemicellulolytic bacteria
AUTHOR: Bergquist P L (Reprint); Gibbs M D; Morris D D; Teo V S; Saul D J; Moran H W
CORPORATE SOURCE: MACQUARIE UNIV, SCH BIOL SCI, N RYDE, NSW 2109, AUSTRALIA (Reprint); UNIV AUCKLAND, SCH MED, DEPT MOL MED, AUCKLAND, NEW ZEALAND; UNIV AUCKLAND, SCH BIOL SCI, CTR GENE TECHNOL, AUCKLAND, NEW ZEALAND; UNIV WAIKATO, THERMOPHILE RES UNIT, HAMILTON, NEW ZEALAND
COUNTRY OF AUTHOR: AUSTRALIA; NEW ZEALAND
SOURCE: FEMS MICROBIOLOGY ECOLOGY, (FEB 1999) Vol. 28, No. 2, pp. 99-110.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0168-6496.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Many thermophilic bacteria belong to groups with deep phylogenetic lineages and ancestral forms were established before the occurrence of eucaryotes that produced cellulose and hemicellulose. Thus they may have acquired their beta-glycanase genes from more recent mesophilic bacteria. Most research has focussed on extremely thermophilic eubacteria growing above 65 degrees C under anaerobic conditions. Only recently have aerobic cellulolytic thermophiles been described from widely separated lineages (for example, Rhodothermus marinus, Caldibacillus cellulovorans). Many thermophilic bacteria produce **cellulases** and xylanases that have novel structures, with additional protein domains not identified with their catalytic activity. Many of these enzymes are multifunctional and code for more than one catalytic activity. This type of enzyme structure was first identified in the extreme thermophile Caldicellulosiruptor caccharolyticus. There is a general relatedness evident between catalytic domains, cellulose binding domains and other ancillary domains, which suggests that there may have been significant lateral gene transfer in the evolution of these microorganisms. Detailed molecular studies show that there is variation in the sequences of these related but not identical genes from taxonomically widely-separated organisms.

L4 ANSWER 16 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1998-08934 BIOTECHDS
TITLE: Glycosidase enzymes from organisms of the genera e.g. Thermotoga, Thermococcus, etc.; recombinant enzyme preparation and use in glucose preparation for the food, pharmaceutical, surfactant and textile industry
AUTHOR: Bylina E J; Swanson R V; Mathur E J; Lam D E
PATENT ASSIGNEE: Diversa
LOCATION: San Diego, CA, USA.
PATENT INFO: WO 9824799 11 Jun 1998
APPLICATION INFO: WO 1997-US22623 8 Dec 1997
PRIORITY INFO: US 1997-56916 10 Oct 1997; US 1996-56916 6 Dec 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-362407 [31]

AB A new nucleic acid encoding protein (I) (protein sequence and DNA sequence or RNA sequence specified) can be contained on a vector and used to transform a host cell for production of recombinant (I). (I) is used to produce glucose from soluble cell oligosaccharides in e.g. waste, food, feed or surfactants, for use in food, pharmaceutical, textile and surfactant industrries. (I) is preferably a glycosidase from Desulfurococcus sp. M11TL, Thermotoga sp. OC1/4V-33BG, Thermotoga **maritima** MSB8 or MSB8-6GP2, Staphylococcus marinus F1-12G, Thermococcus sp. 9N2-31B/G, Thermococcus alcaliphilus AEDII12RA, Thermococcus chitinophagus GC74-22G, Pyrococcus furiosus VC1-7G1, a **cellulase** (EC-3.2.1.4) from Bankia gouldi 37GP1 or Thermotoga sp. OC1/4V, an alpha-galactosidase (EC-3.2.1.22) from T. **maritima** 6GC2, an endo-1,4-beta-D-mannanase (EC-3.2.1.78) from T. **maritima** 6GP2, a pullulanase (EC-3.2.1.41) from T. **maritima** 6GP2, a beta-mannosidase (EC-3.2.1.25) from AEPII-1a or unidentified protein from T. **maritima** MSB8-6GB4, Pyrococcus furiosus VC1-7EG1 or Bankia gouldi 37GP4. (92pp)

L4 ANSWER 17 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:942580 SCISEARCH

THE GENUINE ARTICLE: 145XB
TITLE: **Purification**, characterization, and molecular analysis of thermostable **cellulases** CelA and CelB from Thermotoga neapolitana
AUTHOR: Bok J D; Yernool D A; Eveleigh D E (Reprint)
CORPORATE SOURCE: RUTGERS STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM, 76 LIPMAN DR, NEW BRUNSWICK, NJ 08901 (Reprint); RUTGERS STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM, NEW BRUNSWICK, NJ 08901
COUNTRY OF AUTHOR: USA
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (DEC 1998) Vol. 64, No. 12, pp. 4774-4781.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two thermostable endocellulases, CelA and CelB, were **purified** from Thermotoga neapolitana. CelA (molecular mass, 29 kDa; pI 4.6) is optimally active at pH 6.0 at 95 degrees C, while CelB (molecular mass,30 kDa; pI 4.1) has a broader optimal pH range (pH 6.0 to 6.6) at 106 degrees C. Both enzymes are characterized by a high level of activity (high V-max value and lent apparent K-m value) with carboxymethyl cellulose; the specific activities of CelA and CelB are 1,219 and 1,536 U/mg, respectively. With p-nitrophenyl cellobioside the V-max values of CelA and CelB are 69.2 and 18.4 U/mg, respectively, while the K-m values are 0.97 and 0.3 mM,respectively. The major end products of cellulose hydrolysis, glucose and cellobiose, competitively inhibit CelA, and CelB, The K-i values for CelA are 0.44 M bp glucose and 2,5 mM far cellobiose; the K-i values far CelB are 0.2 M for glucose and 1.16 mM for cellobiose, CelB preferentially cleaves larger cellooligomers, producing cellobiose as the end product; it also exhibits significant transglycosylation activity, This enzyme is highly thermostable and has half-lives of 130 min at 106 degrees C and 26 min at 110 degrees C. A single **clone** encoding the celA and celB genes was identified by screening a T. neapolitana genomic library in Escherichia coli, The celA gene encodes a 257-amino-acid protein, while celB encodes a 274-amino-acid protein. Both proteins belong to family 12 of the glycosyl hydrolases, and the two proteins are 60% similar to each other, Northern! blots of T. neapolitana mRNA revealed that celA and celB are monocistronic messages, and both genes are inducible by cellobiose and are repressed by glucose.

L4 ANSWER 18 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:313351 SCISEARCH
THE GENUINE ARTICLE: ZH504
TITLE: **Cloning**, sequencing and overexpression of a Rhodothermus marinus gene encoding a thermostable **cellulase** of glycosyl hydrolase family 12
AUTHOR: Halldorsdottir S; Thorolfsdottir E T; Spilliaert R; Johansson M; Thorbjarnardottir S H; Palsdottir A; Hreggvidsson G O; Kristjansson J K; Holst O; Eggertsson G (Reprint)
CORPORATE SOURCE: UNIV ICELAND, INST BIOL, MOL GENET LAB, IS-108 REYKJAVIK, ICELAND (Reprint); UNIV ICELAND, INST BIOL, MOL GENET LAB, IS-108 REYKJAVIK, ICELAND; TECHNOL INST ICELAND, DEPT BIOTECHNOL, IS-112 REYKJAVIK, ICELAND; LUND UNIV, CTR CHEM & CHEM ENGN, S-22100 LUND, SWEDEN
COUNTRY OF AUTHOR: ICELAND; SWEDEN
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (MAR 1998) Vol. 49, No. 3, pp. 277-284.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY

10010.
ISSN: 0175-7598.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A gene library from the thermophilic eubacterium Rhodothermus marinus, strain ITI 378, was constructed in pUC18 and transformed into Escherichia coli. Of 5400 transformants, 3 were active on carboxymethylcellulose. Three plasmids conferring **cellulase** activity were **purified** and were all found to contain the same **cellulase** gene, celA. The open reading frame for the celA gene is 780 base pairs and encodes a protein of 260 amino acids with a calculated molecular mass of 28.5 kDa. The amino acid sequence shows homology with **cellulases** in glycosyl hydrolase family 12. The celA gene was overexpressed in E. coli when the pET23, T7 phage RNA polymerase system was used. The enzyme showed activity on carboxymethylcellulose and lichenan, but not on birch xylan or laminarin. The expressed enzyme had six terminal histidine residues and was **purified** by using a nickel nitrilotriacetate column. The enzyme had a pH optimum of 6-7 and its highest measured initial activity at 100 degrees C. The heat stability of the enzyme was increased by removal of the histidine residues. It then retained 75% of its activity after 8 h at 90 degrees C.

L4 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
ACCESSION NUMBER: 1997:776184 CAPLUS
DOCUMENT NUMBER: 128:72370
TITLE: Endoglucanases gene sequences from thermophilic archael bacteria
INVENTOR(S): Lam, David E.; Mathur, Eric J.
PATENT ASSIGNEE(S): Recombinant Biocatalysis, Inc., USA; Lam, David E.; Mathur, Eric J.
SOURCE: PCT Int. Appl., 125 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9744361	A1	19971127	WO 1997-US8793	19970522
W: AU, CA, IL, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5789228	A	19980804	US 1996-651572	19960522
AU 9732852	A1	19971209	AU 1997-32852	19970522
AU 719444	B2	20000511		
EP 923608	A1	19990623	EP 1997-928650	19970522
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000512842	T2	20001003	JP 1997-542781	19970522
US 6074867	A	20000613	US 1997-951086	19971015

PRIORITY APPLN. INFO.: US 1996-651572 A2 19960522
WO 1997-US8793 W 19970522

AB The invention provides a **purified** thermostable enzyme derived from the archael bacterium AEPII1a. The enzyme has a mol. wt. of .apprx.60.9 kDa and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired. Also included are an addnl. 23 genes and their encoded endoglucanases having homol. to the AEPII1a enzyme. The **cellulases** enzymes may be used for degrdn. of cellulose for the conversion of plant biomass into fuels and chems., for use in detergents, the textile industry, in animal feed, in waste

treatment, and in the fruit juice/brewing industry for the clarification and extn. of juices.

L4 ANSWER 20 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 97:854403 SCISEARCH
THE GENUINE ARTICLE: YF644
TITLE: Characterization of the xylanolytic enzyme system of the extreme thermophilic anaerobic bacteria Thermotoga **maritima**, T-neapolitana, and T-thermarum
AUTHOR: Sunna A; Puls J; Antranikian G (Reprint)
CORPORATE SOURCE: TECH UNIV HAMBURG, ARBEITSBEREICH BIOTECHNOL 1, DENICKESTR 15, D-21073 HAMBURG, GERMANY (Reprint); TECH UNIV HAMBURG, ARBEITSBEREICH BIOTECHNOL 1, D-21073 HAMBURG, GERMANY; BUNDESFORSCH ANSTALT FORST & HOLZWIRTSCH, INST HOLZCHEM, D-21031 HAMBURG, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY A-PHYSIOLOGY, (NOV 1997) Vol. 118, No. 3, pp. 453-461.
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB.
ISSN: 0300-9629.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 44

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The extreme thermophilic anaerobic bacteria Thermotoga **maritima**, T. neapolitana, and T. thermarum when grown on xylan produce extremely thermoactive xylanases, beta-xylosidases and alpha-arabinofuranosidases. Most of these enzymes are active over a broad temperature and pH range, namely between 40 and 110 degrees C and pH 4 to 9. The xylanases are active towards soluble, as well as insoluble xylan. Unlike the enzyme system of T. **maritima** and T. thermarum, the xylanase activity of T. neapolitana is activated (up to 211%) in the presence of Ca2+, Mg2+, Co2+, and Mn2+. The xylanolytic enzyme system of T. thermarum is inactive towards cellulose. Xylan hydrolysis experiments indicate the presence of endoxylanases in the enzyme preparation of all strains investigated, with the main products being xylobiose, xylotriose, and larger xylooligosaccharides. (C) 1997 Elsevier Science Inc.

L4 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 8
ACCESSION NUMBER: 1996:599328 CAPLUS
DOCUMENT NUMBER: 125:268866
TITLE: Analysis of a Thermotoga **maritima** DNA fragment encoding two similar thermostable **cellulases**, CelA and CelB, and characterization of the recombinant enzymes
AUTHOR(S): Liebl, Wolfgang; Ruile, peter; Bronnenmeier, Karin; Riedel, Katrin; Lottspeich, Freidrich; Greif, Ingrid
CORPORATE SOURCE: Inst. Mikrobiol., Tech. Univ. Muenchen, Munich, D-80290, Germany
SOURCE: Microbiology (Reading, United Kingdom) (1996), 142(9), 2533-2542
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant Escherichia coli **clones** displaying thermostable .beta.-glucanase activity were **isolated** from two different gene libraries of the hyperthermophilic bacterium Thermotoga **maritima** MSB8 (DSM 3109), and the nucleotide sequence of a 1,4-.beta.-glucanase gene designated celA was detd. Amino-terminal sequencing of **cellulase** I previously detected in T. **maritima** cells indicated that the CelA gene encodes this .beta.-glucanase, which is now

designated CelA. CelA, which has a calcd. mol. mass of 29,732 Da, was **purified** from a recombinant e. coli strain to apparent homogeneity as judged by SDS-PAGE with a 44% yield. The enzyme was most active against sol. substrates such as mixed-linkage .beta.-glucan and CM-cellulose. CelA displayed remarkable thermostability, which was enhanced in the presence of high concns. of salt. Downstream of the celA gene the authors found a second open reading frame, celB, whose nucleotide sequence was 58% identical to celA. Exptl. proof that celB also encodes a .beta.-glucanase was obtained by sepn. from CelA and expression in E. coli under the control of an efficient host promoter. According to the deduced amino acid sequences, CelB, in contrast to CelA, contains a signal peptide at the amino terminus. CelB and Cela had similar substrate specificities and temp. optima, but differed in their pH optima. Also, the addn. of salt had a less stabilizing effect on CelB than on CelA. Nine 30 bp direct repeats, each itself representing a sequence with imperfect dyad symmetry, were detected upstream of the CelA-celB **cellulase** gene cluster.

L4 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 9
ACCESSION NUMBER: 1995:479619 CAPLUS
DOCUMENT NUMBER: 122:259425
TITLE: **Purification** of Thermotoga **maritima** enzymes for the degradation of cellulosic materials
AUTHOR(S): Bronnenmeier, Karin; Kern, Anja; Liebl, Wolfgang; Staudenbauer, Walter L.
CORPORATE SOURCE: Institut fur Mikrobiologie, Technische Universitat Munchen, Munich, 80 290, Germany
SOURCE: Applied and Environmental Microbiology (1995), 61(4), 1399-407
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A sepn. procedure for the anal. of the enzyme components of the hyperthermophilic bacterium Thermotoga **maritima** involved in cellulose and xylan degrdn. was developed. Resoln. of the enzymes was achieved by a combination of fast-protein liq. chromatog. anion exchange and hydrophobic interactions chromatog. Enzyme fractions were assayed for hydrolysis of Avicel, CM-cellulose (CMC), .beta.-glucan, laminarin, xylan, p-nitrophenyl-.beta.-D-glucoside, p-nitrophenyl-.beta.-D-cellobioside, p-nitrophenyl-.beta.-D-xyloside, p-nitrophenyl-.alpha.-L-arabinofuranoside, and 4-O-methyl-glucuronosyl-xylotriose. The activities of two **cellulases**, one laminarinase, one xylanase, two putative .beta.-D-xylosidases, .alpha.-D-glucuronidase, and .alpha.-L-arabinosidase were identified. Because of their selective retardation of a Superdex gel filtration column, the two **cellulases** could be **purified** to homogeneity. According to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, mol. masses of 27 and 29 kDa, resp., were detd. for **cellulase** I and **cellulase** II. Maximal activities of both enzymes were obsd. at 95 .degree.C between pH 6.0 and 7.5. In the presence of 2.5 M NaCl the **purified** enzymes retained about 90% of their initial activities after a 6-h incubation at 80 .degree.C. On the basis of its activity towards CMC, **cellulase** I was classified as endo-.beta.-1,4-glucanase. **Cellulase** II was able to attack Avicel in addn. to CMC, .beta.-glucan, and p-nitrophenyl-.beta.-D-cellobioside. It releases cellobiose and cellotriose from Avicel. The latter product is further cleaved into glucose and cellobiose. **Cellulase** II may therefore be classified as exo-.beta.-1,4-glucanase.

L4 ANSWER 23 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 94:155837 SCISEARCH
THE GENUINE ARTICLE: MY351
TITLE: DOMAIN-STRUCTURE OF THE ACETOGENIUM-KIVUI SURFACE-LAYER

REVEALED BY ELECTRON CRYSTALLOGRAPHY AND SEQUENCE-ANALYSIS
AUTHOR: LUPAS A; ENGELHARDT H; PETERS J; SANTARIUS U; VOLKER S; BAUMEISTER W (Reprint)
CORPORATE SOURCE: MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY (Reprint); MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: JOURNAL OF BACTERIOLOGY, (MAR 1994) Vol. 176, No. 5, pp. 1224-1233.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The three-dimensional structure of the Acetogenium kivui surface layer (S-layer) has been determined to a resolution of 1.7 nm by electron crystallographic techniques. Two independent reconstructions were made from layers negatively stained with uranyl acetate and Na-phosphotungstate. The S-layer has p6 symmetry with a center-to-center spacing of approximately 19 nm. Within the layer, six monomers combine to form a ring-shaped core surrounded by a fenestrated rim and six spokes that point towards the axis of threefold symmetry and provide lateral connectivity to other hexamers in the layer. The structure of the A. kivui S-layer protein is very similar to that of the Bacillus brevis middle wall protein, with which it shares an N-terminal domain of homology. This domain is found in several other extracellular proteins, including the S-layer proteins from Bacillus sphaericus and Thermus thermophilus, Omp alpha from Thermotoga **maritima**, an alkaline **cellulase** from Bacillus strain KSM-635, and xylanases from Clostridium thermocellum and Thermoanaerobacter saccharolyticum, and may serve to anchor these proteins to the peptidoglycan. To our knowledge, this is the first example of a domain conserved in several S-layer proteins.

L4 ANSWER 24 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 94:150772 SCISEARCH
THE GENUINE ARTICLE: MY168
TITLE: COMPARATIVE AMINO-ACID-SEQUENCE ANALYSIS OF THERMOTOGA-**MARITIMA** BETA-GLUCOSIDASE (BGLA) DEDUCED FROM THE NUCLEOTIDE-SEQUENCE OF THE GENE INDICATES DISTANT RELATIONSHIP BETWEEN BETA-GLUCOSIDASES OF THE BGA FAMILY AND OTHER FAMILIES OF BETA-1,4-GLYCOSYL HYDROLASES
AUTHOR: LIEBL W (Reprint); GABELSBERGER J; SCHLEIFER K H
CORPORATE SOURCE: TECH UNIV MUNICH, LEHRSTUHL MIKROBIOL, ARCISSTR 21, D-80290 MUNICH, GERMANY (Reprint)
COUNTRY OF AUTHOR: GERMANY
SOURCE: MOLECULAR & GENERAL GENETICS, (JAN 1994) Vol. 242, No. 1, pp. 111-115.
ISSN: 0026-8925.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 21

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The primary structure of the bglA gene region encoding a beta-glucosidase of Thermotoga **maritima** strain MSB8 was determined. The bglA gene has the potential to code for a polypeptide of 446 amino acids with a predicted molecular mass of 51545 Da. The T. **maritima** beta-glucosidase (BglA) was overexpressed in E. coli at a level comprising approximately 15-20% of soluble cellular protein. Based on its amino acid sequence, as deduced from the nucleotide sequence of the gene, BglA can be classified as a broad-specificity beta-glucosidase and as a member of the beta-glucosidase family BGA, in agreement with the results of enzymatic characterization of the recombinant protein.

Comparative sequence analysis revealed distant amino acid sequence similarities between BGA family beta-glucosidases, a beta-xylosidase, beta-1,4-glycanases of the enzyme family F (mostly xylanases), and other families of beta-1,4-glycosyl hydrolases. This result indicates that BGA beta-glucosidases may comprise one enzyme family within a large 'enzyme order' of retaining beta-glycosyl hydrolases, and that the members of these enzyme groups may be inter-related at the level of active site architecture and perhaps even on the level of overall three-dimensional fold.

L4 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 10
ACCESSION NUMBER: 1995:225667 CAPLUS
DOCUMENT NUMBER: 122:154681
TITLE: **Cellulase** and xylanase systems of Thermotoga neapolitana
AUTHOR(S): Bok, Jin Duck; Goers, Steven K.; Eveleigh, Douglas E.
CORPORATE SOURCE: Biochem. and Microbiol., Rutgers Univ., New Brunswick, NJ, 08903-0231, USA
SOURCE: ACS Symposium Series (1994), 566(Enzymatic Conversion of Biomass for Fuels Production), 54-65
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 67 refs. Thermotoga neapolitana and T. **maritima**, hyperthermophiles that grow optimally at 80.degree.C, have very similar **cellulase** systems based on SDS-PAGE/zymogram anal. T. neapolitana has at least three types of **cellulase** components, including an aryl .beta.-glucosidase (BglA), a .beta.-glucosidase with cellobiohydrolase activity (BglB) and endoglucanase (EndoA and B). The Sephadex adsorption properties of EndoB allowed a facile **purifn.** from both T. neapolitana and T. **maritima**. All enzymes exhibited very high thermostability, e.g. EndoB t1/2=8 h at 100.degree.C. The specific activities (CMC U/mg) of both endoglucanases are very high: 1,200 for EndoA and 1,500 for EndoB. These properties make Thermotoga endoglucanases potentially useful for industrial application. To facilitate prodn., these endoglucanase genes and those of BglA and B have been **cloned** in E. coli. Furthermore, an endo-acting .beta.-1,4-xylanase from T. neapolitana was discovered and found to have remarkable thermal stability (t1/2=130 h at 82.degree.C), and pH and temp. optima resp. of 5.5 and 85.degree.C. This xylanase has little endoglucanase activity and has potential application for biol. bleaching in the pulp and paper industry.

L4 ANSWER 26 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1992-09382 BIOTECHDS
TITLE: **Cloning** and characterization of an endoglucanase and a xylanase from the hyperthermophilic eubacterium Thermotoga **maritima**;
cellulase complex characterization and thermostable CM-**cellulase** gene **cloning** and expression (conference abstract)
AUTHOR: Bok J D; Goers S K; Eveleigh D E
LOCATION: Rutgers University, New Brunswick, NJ 08903, USA.
SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1992) 92 Meet., 312
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cellulose and hemicellulose biomass is a central energy reserve for many ecological systems. Thermotoga **maritima**, an anaerobic, hyperthermophilic (growth at 90 deg) marine eubacterium is cellulolytic. The hydrolases of this bacterium have been characterized, and include 1 endoglucanase (CM-**cellulase**, EC-3.2.1.4), up to 3 endo-1,4-beta-D-xylanases (EC-3.2.1.8), 1 beta-glucosidase (EC-3.2.1.21)/cellobiohydrolase (EC-3.2.1.91) and 1 beta-glucosidase.

The CM-**cellulase** was **purified** to homogeneity and characterized. It had a temp. optimum of 90-95 deg at pH 5, with specific activity of 130 U/mg. It was a small (mol.wt. 26,000) acidic enzyme with an isoelectric point at 3.6. A gene bank from T. **maritima** was constructed in Escherichia coli using plasmid pUC119. Gene egI encoding **cellulase** was **cloned** and expressed. The gene product was **purified** and characterized. **Cloned** and native enzymes had almost identical characteristics. 1 Xylanase has also been **cloned** and characterized. (0 ref)

L4 ANSWER 27 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1991-07888 BIOTECHDS
TITLE: **Cloning** genes from thermophiles: a review of current research; (conference paper)
AUTHOR: McHale R H; Saul D; Ashby M; Luthi E; Whitefield J; Bergquist P L
LOCATION: Thermophile Genetics Research Group, Centre for Gene Technology, Department of Cellular and Molecular Biology, University of Auckland, New Zealand.
SOURCE: Ferment.Technol.Ind.Appl.; (1990) 9-14
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **cloning** of genes from thermophilic microorganisms was reviewed. A number of genes encoding thermostable enzymes involved in degradation of cellulose and xylan have been **cloned**, sequenced and expressed in Escherichia coli, and in some cases, in Bacillus subtilis. These include the beta-glucosidase (EC-3.2.1.21), **cellulase** (EC-3.2.1.4), cellobiohydrolase (EC-3.2.1.91), endo-1,4-beta-D-xylanase (EC-3.2.1.8) and beta-xylosidase (EC-3.2.1.37) genes of Caldocellum saccharolyticum. The pullulanase (EC-3.2.1.41) gene of this thermophilic bacterium has also been **cloned**, sequenced and expressed in E. coli. The ease of expression of the genes in E. coli has not been found for genes from other thermophiles, such as Thermotaga **maritima**. Alternative strategies have therefore been adopted and include the use of the polymerase chain reaction to amplify specific gene sequences, immunological assays and the development of a gene transfer system in Thermus sp. Genes for 16S ribosomal RNAs from thermophiles have been **cloned** and sequenced as an aid to taxonomic and phylogenetic investigations. (23 ref)

L4 ANSWER 28 OF 29 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2003)
ACCESSION NUMBER: 83:62865 AGRICOLA
DOCUMENT NUMBER: IND83051918
TITLE: **Purification** of three **cellulases** from the xylophageous larvae of Ergates faber (Coleoptera:Cerambycidae) Pinus **maritima**.
AUTHOR(S): Chararas, C.; Eberhard, R.; Courtois, J.E.; Petek, F.
AVAILABILITY: DNAL (QL495.A1I57)
SOURCE: Insect biochemistry., 1983 Vol. 13, No. 2. p. 213-218
Publisher: Oxford : Pergamon Press.
ISSN: 0020-1790
NOTE: Includes references.
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

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ACCESSION NUMBER: 1975:404972 CAPLUS
DOCUMENT NUMBER: 83:4972
TITLE: Production and activity of cellulolytic enzymes by

higher fungi from marine and brackish waters

AUTHOR(S): Schaumann, K.
CORPORATE SOURCE: Inst. Meeresforsch., Bremerhaven, Fed. Rep. Ger.
SOURCE: Marine Biology (Berlin, Germany) (1974), 28(3), 221-35
CODEN: MBIOAJ; ISSN: 0025-3162
DOCUMENT TYPE: Journal
LANGUAGE: German

AB Twenty **isolations** of higher marine fungi were examd. in regard to their cellulolytic capabilities. Application of the viscosimetric method produced detailed information on the cellulolytic activities of these fungi. However, only the Cx-component of the total cellulose complex could be estd. by this method. The most active species were Dendryphiella salina, Chaetomium ramipilosum, Asteromyces cruciatus, and Humicola alopallonella. Only very slight or no Cx-activity was obsd. in Cirrenalia macrocephala, Monodictys pelagica, and Zalerion maritimum. These findings reveal no correlation between the cellulolytic activity of the fungi in vitro and their frequency on wood substrates in situ. For example, differences in the intensity of Cx-**cellulase** prodn. of D. salina were caused by variations in salinity and compn. of the nutrient broth, especially by the kind of cellulose added for enzyme induction, and by the addn. or absence of glucose. Most of the Cx-**cellulase** produced was present in the cell-free culture-filtrate. Only a small quantity was absorbed by the cellulose particles or the fungal mycelia. Parallel to the mycelial growth, and accompanied by a shift in pH, the Cx-**cellulase**-activity rose continuously, attaining a max. after several weeks. During further cultivation, the max. remained more or less const. for a long period. Tests using different methods, e.g. cellulose powder-agar plates, proved unsuitable because of spreading hyphal growth, dark pigmentation, heavy sexual or asexual sporulation, and relatively low **cellulase** prodn. during short-culture periods.